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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/714,579	11/13/2003	Xianqiang Li	26757-717.201	5749

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EXAMINER	
SHAW, AMANDA MARIE	
ART UNIT	**PAPER NUMBER**
1634	

DATE MAILED: 03/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/714,579	LI ET AL.	
	Examiner	Art Unit	
	Amanda M. Shaw	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-83 is/are pending in the application.

4a) Of the above claim(s) 74-83 is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-73 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 13 November 2003 is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group I in the reply filed on January 23, 2006 is acknowledged. Accordingly, Claims 1-73 have been examined herein.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 40-47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in that the preamble of the method and the final step do not agree. Claims 40-47 relate to methods for detecting nucleotide sequence variation. The steps listed in the methods do not result in the detection of nucleotide sequence variation. Therefore, it is unclear if the steps listed can achieve the goal of the method.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-22, 24-33, 36, 38-39, 42, 44-45, 49-58, 60-63, and 65 rejected under 35 U.S.C. 103(a) as being unpatentable over Yang et al (PG Pub 2002/0042061) in view of Fishel et al (U.S. Patent 6333153).

Regarding Claim 1, Yang et al teach a method for detecting a nucleotide sequence variation of a target nucleic acid relative to that of a reference nucleic acid, comprising the steps of: providing a Holliday junction structure formed between a target nucleic acid and a reference nucleic acid, forming a complex between the Holliday junction structure and a Holliday junction-binder. Specifically Yang et al teach a method for the detection of a difference between two related nucleic acid sequences (i.e. a reference sequence and a target sequence) by determining whether constructs comprising the sequences are capable of forming a stabilized Holliday junction. The stabilized Holliday junction is detected by one or more binding proteins capable of specifically binding a Holliday junction (Para 0057). The binding proteins used by Yang et al include any molecule or molecules known to those of skill in the art to specifically bind Holliday structures (i.e. RuvA, RuvC, RuvB, RusA, RuvG) (Para 0089).

Yang et al do not specifically teach a method wherein the complex (Holliday junction structure and binder) is contacted with a receptor. However, Yang et al suggest that the detection of a complex comprising a Holliday junction and Holliday junction binding protein can be accomplished by means of an ELISA assay (Para 0102).

It was conventional in the art at the time the invention was made to use antibodies in order to detect proteins bound to DNA. For example Fishel et al teach the use of antibodies to detect DNA double stranded binding proteins (i.e. mutS) bound to a

target DNA. Specifically Fishel et al teach that an antibody bound to a solid support can bind to a complex comprising mutS and DNA (Column 21, 35-52).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Yang et al so as to have contacted the Holliday junction with a receptor (i.e. antibody) bound to a support prior to detection. One skilled in the art would have been motivated to do this for the added benefit of providing an effective means for detecting the binder-Holliday junction complex and of providing a method in which the target (Holliday junction) becomes immobilized so that unbound reagents could be rinsed away with wash steps prior to detection of the Holliday junction, thereby increasing the specificity of the detection assay.

Regarding Claims 2, 13, and 49, Yang et al teach that the target nucleic acid is derived from a targeted region of a test nucleic acid contained in a sample. Specifically Yang et al teach that the reference sequence is typically a polynucleotide of substantially known sequence, and the target sequence is a related sequence for which it is desired to detect whether there is a difference relative to the reference sequence (Para 0059).

Regarding Claims 3, 14, and 50, Yang et al teach that the target nucleic acid is PCR-amplified from a region of the test nucleic acid containing a SNP. Specifically Yang et al teach that to determine if there is any difference between the two related DNA sequences, duplex A and B are amplified, either separately or jointly, by standard PCR using a common set of primers (Para 0068). Typically the target nucleic acid is

different from the reference by either a substitution, deletion or insertion variation or mutation, such as but not limited to a single nucleotide polymorphism (SNP) (Para 0060).

Regarding Claims 4, 15, and 51, Yang et al teach that test nucleic acid is selected from the group consisting of double-stranded test DNA, single-stranded test DNA, test RNA, test DNA-RNA hybrid of a target gene, chromosome, plasmid, or genome of a biological material. Specifically Yang et al teach that the nucleotide may be DNA, RNA, cDNA, DNA-RNA, hybrid or any mixture of the same, and may exist in a double-stranded, single-stranded or partially double-stranded form (Para 0036).

Regarding Claims 5, 16, and 52 Yang et al teach a method wherein the biological material is selected from the group consisting of bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, and humans. Specifically Yang et al teach that nucleic acids of the invention include both nucleic acids and fragments thereof, in purified or unpurified forms, including genes, chromosomes, plasmids, the genomes of biological material such as microorganisms, e.g., bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, humans, and the like (Para 0036).

Regarding Claims 6, 17, and 53, Yang et al teach a method wherein the sequence of the reference nucleic acid differs from the sequence of the target nucleic acid in a single nucleotide position. Specifically Yang et al teach that the difference between the reference nucleic acid and the target nucleic acid can be a substitution, deletion or insertion of any single nucleotide or a series of nucleotides within a sequence (Para 0049).

Regarding Claims 7, 18, and 54, Yang et al teach a method wherein the target nucleic acid is double-stranded. Specifically Yang et al teach that the nucleotide may be DNA, RNA, cDNA, DNA-RNA, hybrid or any mixture of the same, and may exist in a double-stranded, single-stranded or partially double-stranded form (Para 0036).

Regarding Claims 8-9, 19-20, and 55 and 56, Yang et al teach a method wherein the target nucleic acid and the reference nucleic acid both comprise a combination of Target-Tail-1 polynucleotide (made up of a first region that is complementary to the target region of test DNA and a second region designated Tail-1) and Target-Tail-2 polynucleotide (made up of a first region that is complementary to the target region of test DNA and a second region designated Tail-2). The sequence of Tail-1 and that of Tail-2 differ in one or more nucleotide positions. Specifically, Yang et al teach that the target sequence and the reference sequence are prepared as a pair of sequences that are capable of forming a partial duplex. A partial duplex comprises a complementary duplex region and one or more tail regions. A complementary duplex region comprises a target sequence or a reference sequence annealed to its complement. In some case a partial duplex has one tail region and in other cases a partial duplex has two tail regions (Para 0061 and Fig 1).

Regarding Claims 10-11, 21-22, and 57-58, Yang et al teach a method wherein the Target-Tail-1 polynucleotide or the Target-Tail-2 polynucleotide of the target nucleic acid and the Target-Tail-1 polynucleotide or the Target-Tail-2 polynucleotide of the reference nucleic acid are double stranded (Figure 1).

Regarding Claims 23 and 59, Yang et al teach PCR-amplifying the targeted region of the test nucleic acid using a forward primer for the targeted region of the test nucleic acid, a first reverse primer for the targeted region of the test nucleic acid further comprising the sequence of Tail-1, and a second reverse primer for the targeted region of the test nucleic acid further comprising the sequence of Tail-2. Specifically Yang et al teach that the target and reference nucleic acids are amplified by standard PCR using a common set of primers made up of one or more forward primers and two reverse primers. (Fig 1 and Para 0068). The two reverse primers can either share the same 3' end that hybridizes to the same part of template DNA or the 3' end of R1 and R2 can hybridize to different parts of the template DNA.

Regarding Claim 12, Yang et al teach a method for detecting nucleotide sequence variations between a target nucleic acid and a reference nucleic acid, by subjecting the mixture of target nucleic acid and the reference nucleic acid to branch migration conditions such that a Holliday junction structure forms. Specifically Yang et al teach that the target nucleic acid and a reference nucleic acid are contacted under conditions in which they are capable of forming a four-way nucleic acid complex with a branch structure that is capable of migration. Under the contact conditions, if the reference nucleic acid and target nucleic acid are identical, branch migration is capable of going to completion resulting in complete strand exchange (Para 0010). If the reference nucleic acid and target nucleic acid are not identical, branch migration will not occur and a Holliday junction will form.

Regarding Claim 24, 25, 26, 60, 61, and 62, Yang et al teach a method in which the reference nucleic acid sequence or the target nucleic acid has a length of 35-300 base pairs (Para 0107).

Regarding Claims 27, 33, and 63 Yang et al teach a method wherein the step of detecting the presence of the Holliday junction structure in a second complex includes detecting the presence of one or more strands of the Holliday junction by a method selected from the group consisting of colorimetric detection, fluorescence detection, chemiluminescent detection, enzymatic reaction, gel electrophoresis, mass spectroscopy, and oligonucleotide array. Specifically Yang et al teach that the method employs at least one labeled protein to detect the Holliday junction. The label can be endogenous to the protein, e.g., the natural fluorescence of a protein resulting from the fluorescence of amino acids such as tryptophan, tyrosine, or phenylalanine, or a protein side-chain capable of reacting in a detectable manner. The label can be attached to the protein, either during translation or post-translationally. Suitable labels include, but are not limited to, fluorescent molecules (including, for example, fluorescein, rhodamine, and fluorescent proteins and peptides such as GFP and GFP variants and analogs), radioactive groups, solid surfaces, oligonucleotides, enzymes, dyes, chemiluminiscent groups, coenzymes, enzyme substrates, ligands, receptors and small organic molecules (Para 0093). Therefore the method of the detection used by Yang is fluorescence detection.

Regarding Claim 28, as discussed above, Yang does not teach detecting the binder- Holliday junction complex using an immobilized antibody. However, Fishel et al

teach a method for detecting a DNA double stranded binding protein using a receptor (antibody) that is immobilized to a substrate (Column 21, lines 35-52). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Yang so as to have used an immobilized antibody because this would have provided an effective means for detecting the binder-Holliday junction complex and for separating the complex from unbound reagents to thereby increase the specificity of the detection assay.

Regarding Claims 29, 30, 36-37, 42-43, and 65-67, Yang does not teach immobilization of the reagent which detects the binder-Holliday junction on to a solid support. However, Fishel et al teach a method for detecting a DNA double stranded binding protein using a receptor (antibody) that is immobilized to a solid support selected from the group comprising a microsphere bead, a magnetic bead, a well of a culture plate, glass, membrane or fabric. Specifically Fishel et al teach that the solid support can be latex and other polymeric beads, particles, plates, supports, chromatography media, implants, drug delivery vehicles, metal and glass surfaces, gelatinous surfaces such as agarose, alginates, and polyacrylamides, and the like (Column 22, lines 1-8). Fishel et al do not specifically teach that the plates are culture plates, microplates or 96 well plates however it would be obvious to one skilled in the art that the solid support could be any of these. The advantage of using a 96 well plate would be a higher throughput assay.

Regarding Claim 31, 38, and 44, Yang et al teach a method further comprising the step of: isolating the second complex before the step of detecting the presence of

the Holliday junction structure in the second complex. Specifically Yang et al teach that the Holliday junction may be isolated from other molecules in the mixture such as duplexes and single stranded polynucleotides (Para 109).

Regarding Claim 32, 39, and 45, Yang et al teach a method wherein the step of isolating includes a method selected from the group consisting of immunoprecipitation, gel electrophoresis, affinity chromatography, oligonucleotide array and flowing fluid sorting. Specifically Yang et al teach that the Holliday junction may be isolated from duplex DNA and isolated by means of gel electrophoresis, capillary electrophoresis or chromatography (Para 109).

4. Claims 34-35, 40-41, 46-48, 64, 68-71, and 73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yang et al (PG Pub 2002/0042061) in view of Fishel et al (U.S. Patent 6333153) further view of Lishanski (U.S. Patent 6013439).

The teachings of Yang et al and Fishel et al are presented above.

Regarding Claims 34, 35, 40, 41, 46, 47 and 64, the combined references do not teach contacting a target nucleic acid is labeled with a tag selected from the group consisting of biotin, digoxigenin, fluorescent molecule, chemiluminescent moiety, coenzyme, enzyme substrate, radio isotopes, a particle, nucleic acid-binding protein, and polynucleotide.

However, Lishanski et al teach that one of strands (either the target or reference) strand is labeled with a reporter molecule. Any reporter molecule that is detectable can be used. The reporter molecule can be isotopic or nonisotopic, usually non-isotopic, and

can be a catalyst, such as an enzyme, dye, fluorescent molecule, chemiluminescer, coenzyme, enzyme substrate, radioactive group, a particle such as latex or carbon particle, metal sol, crystallite, liposome, cell, etc., which may or may not be further labeled with a dye, catalyst or other detectable group, and the like. The reporter group can be a fluorescent group such as fluorescein, a chemiluminescent group such as luminol, a terbium chelator such as N-(hydroxyethyl) ethylenediaminetriacetic acid that is capable of detection by delayed fluorescence, and the like (Column 15, lines 20-42). Although Lishanski does not teach when the labeling occurs (i.e. after the formation of the 1st or 2nd complex), the ordinary artisan would have recognized that labeling after the formation of the first or second complex would have provided equally effective times to label the strands fo the Holliday structure.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Yang et al so as to have detected a label bound to the Holliday junction to determine the presence of the Holliday junction in order to have achieved the benefits set forth by Lishanski which include having a sensitive, simple, inexpensive way for detecting differences in nucleic acids. The method of Lishanski minimizes the number and complexity of steps and reagents and is suitable for large scale population screening (Column 2, lines 55-60).

Regarding Claim 48, Fishel et al teach that it is an antibody that specifically binds to the protein that is bound to the Holliday junction. Fishel et al teach the use of antibodies to detect DNA double stranded binding proteins (i.e. mutS). Specifically

Fishel et al teach that an antibody bound to a solid support can bind to a complex comprising MutS.

Regarding Claim 68 and 69 Lishanski et al teach that the tag is biotin and the method further comprises: contacting the second complex with an agent that comprises streptavidin conjugated to an enzyme wherein the enzyme is selected from the group consisting of alkaline phosphatase, perioxidase, and urease (Column 25, lines 25-30).

Regarding Claims 70 and 71 Yang et al teach a method wherein wherein the protein that specifically recognizes a Holliday junction is a resolvase or recombinase selected from the group consisting of RuvA, RuvC, RuvB, RusA, RuvG, Cce1 and spCcel from yeast, and Hjc from Pyrococcus furiosusa (Para 0089).

Regarding Claim 73, Fishel et al teach that the antibody being used was a polyclonal antibody (Example 5, lines 46-59).

5. Claim 72 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yang et al (PG Pub 2002/0042061) in view of Fishel et al (U.S. Patent 6333153) in further view of Lishanski (U.S. Patent 6013439) and D' Elia et al (PG Pub 2002/0039761.

The teachings of Yang, Fishel, and Lishanski are presented above. The combined references do not teach that the protein that recognizes the Holliday junction is fused with a his-tag.

However, D' Elia et al teach the use of his-tags fused with proteins. Specifically D' Elia teach that a his-tag is a polyhistidine sequence that binds to columns easily thus allowing for rapid purification of the fusion protein (Para 0031).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Yang et al so as to have used a protein fused with a his-tag to bind to the Holliday junction. It is well known in the art that his-tags are a valuable tool when it comes to protein purification. One would be motivated to use a protein fused with a his tag to achieve the benefits set forth by D' Elia which include rapid protein.

Conclusion

6. No Claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amanda M. Shaw whose telephone number is (571) 272-8668. The examiner can normally be reached on Mon-Fri 7:30 TO 4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached at 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Amanda M. Shaw
Examiner
Art Unit 1634
March 6, 2006

CARLA J. MYERS
PRIMARY EXAMINER